Volume 13 Number 23 1985 Nucleic Acids Research

The nucleotide sequence of tobacco rattle virus RNA-2 (CAM strain)

Sandra T.Bergh, Michael G.Koziel[1], Shu-Ching Huang[2], Robert A.Thomas, David P.Gilley and Albert Siegel*

Department of Biological Sciences, Wayne State University, Detroit, MI 48202, USA

Received 21 October 1985; Accepted 30 October 1985

ABSTRACT

The nucleotide sequence of the smaller genomic strand (RNA-2) of the bipartite tobacco rattle virus (CAM strain) has been determined. RNA-2 is capped at the 5' terminus and contains 1799 nucleotide residues. There is a single 223 codon long open reading frame extending from nucleotide 574 to 1242 which designates a protein of Mr 23,654. The derived amino acid composition, in percent, matches that previously determined for the virus capsid protein. The long open reading frame is flanked by 5' and 3' untranslated regions of 573 and 554 nucleotides, respectively. The 5' leader sequence contains two different sets of direct repeats, one of 119 nucleotides and the other of 76. It also contains 13 apparently unused AUG codons, four of which lie in the same frame as the capsid protein cistron. The 3' terminal sequence of RNA-2 is identical to that of the larger genomic strand (RNA-1) for 459 nucleotides.

INTRODUCTION

Tobacco rattle virus (TRV) is a positive-stranded RNA virus with a bipartite genome. The smaller of the two genomic RNAs, RNA-2, varies in size from Mr 0.7 to 1.4 $x10^6$ depending upon the strain and is capped at the 5' end. The larger RNA, RNA-1, has a molecular weight of about 2.4 x 10^6 in all strains of TRV. The 3' termini of RNAs 1 and 2 are not polyadenylated and there is no evidence that they can be aminoacylated. A single species of capsid protein (Mr 23,000, CAM strain) separately encapsidates RNA-1 and RNA-2 into rods of two lengths. The longer rod, or its RNA-1, can initiate an infection in which the infectious agent is a replicating RNA which spreads from cell to cell. However, no nucleoprotein particles are produced. The shorter rod, or its RNA-2, is not by itself infectious but when included in an inoculum with the longer rod or RNA-1, allows the production of rods of both lengths. Thus, it is presumed that RNA-1 contains the information necessary for RNA replication and cell-to-cell movement and that RNA-2 codes for the capsid protein (reviewed in 1).

In vitro translation studies have indicated that the TRV genome can code for at least four proteins. RNA-1 directs the synthesis of two pro-

teins initiating from the same point, a 120K protein and a readthrough product of 170K (2). The protein translated from RNA-2 has been confirmed as capsid protein on the basis of coelectrophoresis, specific aggregation with capsid protein, and coincidence of tryptic peptide fingerprints (3,4). A fourth protein of about 30K is translated from a subgenomic RNA species. The cistron for this protein has alternatively been assigned to both RNA-1 and RNA-2 (2, 4, 5). We have cloned and sequenced TRV CAM strain RNA-2 to determine its organization and properties. It is the smallest RNA-2 (Mr 0.7 x 10^6) known for any TRV strain. Our results show that RNA-2 contains only one large open reading frame flanked by long 5' leader and 3' untranslated sequences, and thus contains information only for capsid protein.

MATERIALS AND METHODS

Virus Purification

Tobacco rattle virus (strain CAM) was propagated in Christie's Nicotiana hybrid (6). Virus was purified by a modification of the procedure of Hari (7). Leaf tissue was blended with one volume of 10 mM citrate-phosphate, pH 7.4, containing 0.5% 2-mercaptoethanol. The macerate was filtered through two layers of cheesecloth and centrifuged for 20 min at 16,000 x g and 4°C. The supernatant solution was made to 1.75% NaCl and 4% polyethylene glycol 6000, stirred at 4°C for 3 to 16 hours, and centrifuged for 10 min at 16,000 x g and 4°C. Viral pellets were resuspended in a minimum volume of 20 mM Tris, pH 7.4, 2 mM $MgCl_2$, 1% 2-mercaptoethanol. CsCl was added, 4.2 g for every 12 ml of suspension, and the mixture was centrifuged in a Beckman Ti 50 rotor for 20 hours at 45,000 rpm. Banded virus was collected and dialyzed against several changes of 20 mM Tris, pH 7.4, 2 mM $MgCl_2$.

Extraction and Purification of RNA-2

The virus suspension was brought to 40 mM Tris, 5 mM acetic acid, 1 mM EDTA, pH 8.2 (TAE) and 1.9% sodium dodecyl sulfate and extracted with an equal volume of buffer saturated phenol and ½ volume of 24:1 chloroform:octanol. The aqueous phase was reextracted with an equal volume of chloroform:octanol, and the RNA was ethanol precipitated, pelleted and resuspended in water.

The viral RNA was subjected to electrophoresis through 1% agarose either with TAE buffer or with BE buffer (40 mM Borate, 1 mM EDTA, pH 8.2) in the presence of 5 mM methyl mercuric hydroxide (8). RNAs 1 and 2 were recovered by electroelution, sequential extraction with equal volumes of

25:24:1 phenol:chloroform:octanol and 24:1 chloroform:octanol, and ethanol precipitation.

cDNA Synthesis and Cloning

Gel purified RNA-2 was polyadenylated using Sippel's B reaction (9). To inhibit ribonuclease activity, the reaction was modified at times by incubation at 15° in the presence of 1 mM EGTA and 3 mM 2'-3' adenosine monophosphate. At other times 0.5 U/µl human placental RNAse inhibitor (Promega) and dithiothreitol to 2 mM were added.

Double stranded cDNA was synthesized and cloned by G-C tailing into the Pst I site of pBR325 or PBR322 essentially according to the procedure of Land (10). However, in our initial set of cDNA reactions, second strand cDNA synthesis was primed by hairpin formation at the 3' terminus of the first strand and catalyzed with DNA polymerase large fragment, after which the hairpin was opened with S1 nuclease (11). In later reactions first strand cDNA was enriched for full length material by sucrose gradient centrifugation before tailing and second strand synthesis. In a modification of the procedure of Crouse (12), C-tailed double stranded cDNA, as well as Pst I cut and G-tailed pBR322, were purified on low melting agarose gels, mixed and directly annealed in the presence of annealing salts (10). E. coli HB101 cells were transformed with the resulting chimeric plasmids and screened for inserts of TRV RNA-2 by colony filter hybridization (13) using as a probe random-primed cDNA to RNA-2 (14). Plasmids from colonies giving strong signals were purified and mapped for restriction endonuclease sites.

cDNA Sequencing

Inserts, or fragments thereof, released from the selected chimeric plasmids and purified on agarose gels (15) were subcloned into M13mp8 or M13mp9 for sequence determination by the Sanger dideoxynucleotide method (16). Both [α-^{32}P]dCTP, as well as [^{35}S]-dATPαS were used as radioactive label. Buffer gradient gels were used in ^{35}S sequencing reactions (17). Sequencing data were stored and analyzed with the aid of the Larson and Messing sequence analysis program (18).

RNA Sequencing

Portions of TRV RNA-2 were sequenced directly in order to confirm the cDNA derived sequence . The 3' terminus was end-labelled with [5'^{32}P]pCp and sequenced by partial enzymatic digestion (19,21). The 5' terminus was chemically decapped (21), end-labelled with [α-^{32}P]GTP using vaccinia guanylyl transferase (22), and sequenced as above. Several internal regions were sequenced by the dideoxynucleotide method using RNA-2 or RNA-1 direct-

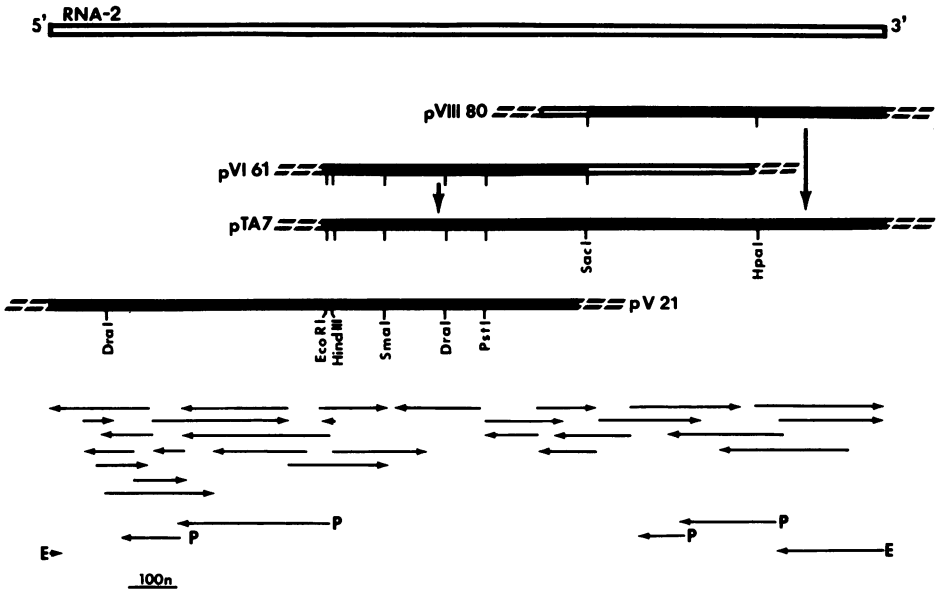


Fig. 1: TRV RNA-2 cDNA clones and the nucleotide sequencing strategy. Below a representation of RNA-2 are four cloned RNA-2 cDNAs shown as open and closed bars with partial adjoining plasmid segments denoted by dashed bars. Plasmid pTA7 was constructed from pVIII80 and pVI61 to join the cDNA regions represented by filled bars. The length and direction of sequences obtained from M13 subclones of pTA7 and pV21 are shown as thin arrows. Sequence data obtained from RNA-2 templates and oligonucleotide primers are indicated by arrows originating from "P"s. The two 3' most primers were also used for the partial sequencing of TRV RNA-1. Arrows originating from "E"s represent sequences obtained by partial enzymatic digestion of end-labelled RNA-2.

ly as template and synthetic pentadecamer oligonucleotides as primers for reverse transcription. These oligomers were either synthesized manually using the New England Biolabs DNA Synthesis kit or supplied by Rockefeller University or Beckman, Inc.

RESULTS

Strategy

Enzymatically polyadenylated TRV RNA-2 was used as template to direct synthesis of double stranded cDNA fragments which were cloned into either plasmid pBR325 or pBR322 by G-C tailing. Two of the pBR325 clones, pVI61 and pVIII80, and one pBR322 clone, pV21, were chosen for further analysis. The pVI61 and pVIII80 inserts were found to contain an overlapping region (Figure 1) and were joined in the proper orientation at a common SacI site

and the plasmid BamHI site to form pTA7. The sequences of the inserts contained within plasmids pTA7 and pV21 were determined and together these correspond to the entire length of RNA-2 (Figure 1). The only difference found between the cDNA derived sequence and that obtained directly from regions of the RNA was that the cDNA contained an additional thymidine residue at the 5' terminus that was not observed in the RNA. Because the 5' terminal sequence of RNA-2 is 5'AAAATTTT..., the additional thymidine could have been added during hairpin formation of the initial cDNA strand by reverse transcriptase. S1 nuclease was not used to open such hairpins during generation of pV21. Instead, the first cDNA strand was C-tailed and oligo (dG) was used as primer for second strand synthesis.

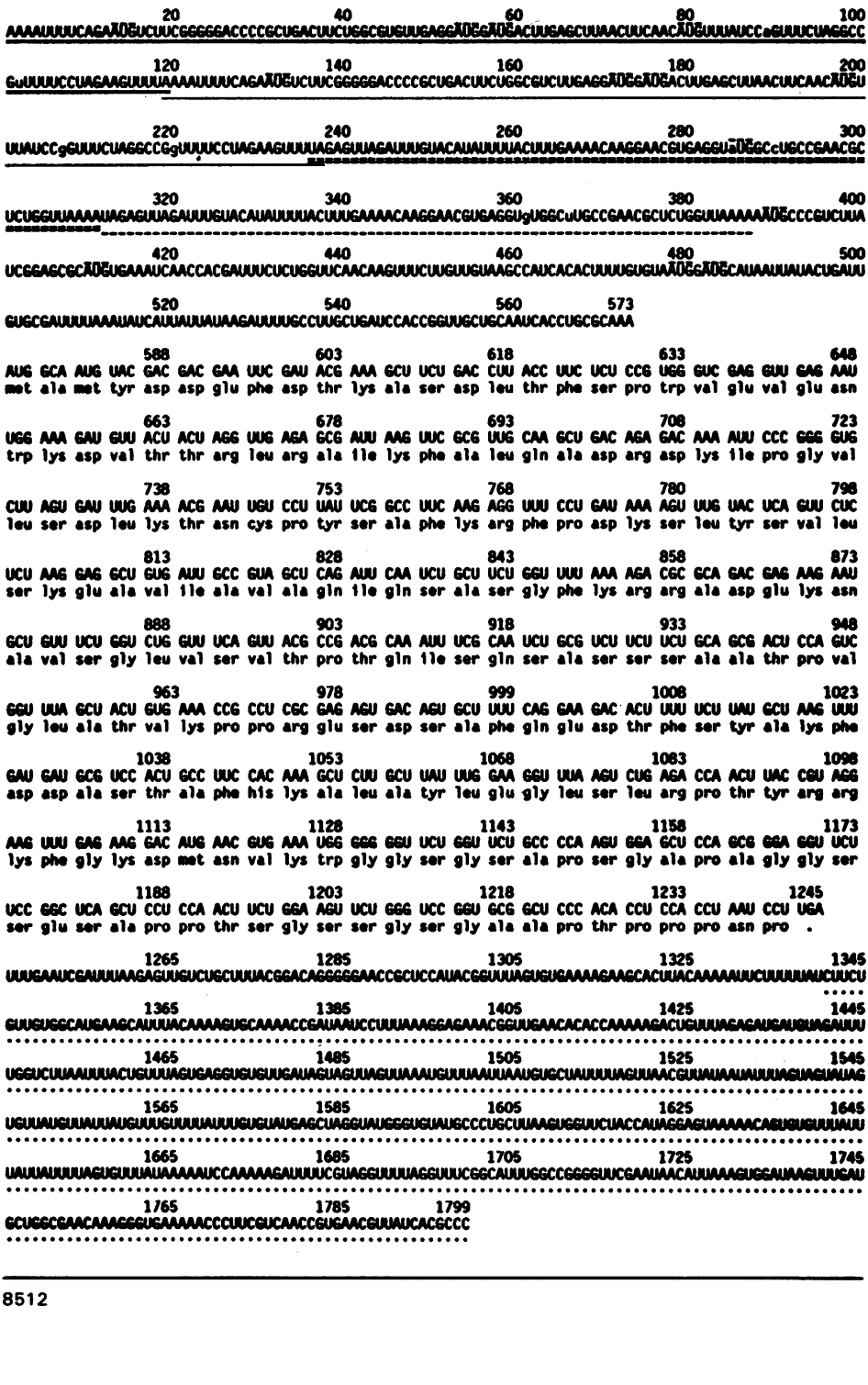
The Large Open Reading Frame

Figure 2 shows the complete sequence of the 1799 nucleotide TRV RNA-2 (CAM strain). Its base composition, which agrees exactly with that previously published (23), is shown in Table 1. A computer search for all possible initiation and termination codons in both the viral RNA strand and its complement shows only one large open reading frame. The methionine residue at position 574 could initiate translation of a 223 amino acid polypeptide (Mr 23,654) terminating at nucleotide 1243. The amino acid composition closely matches that determined for the capsid protein in two independent investigations (Table 2; Ref. 24, 25). The two previous studies assumed the number of amino acid residues in CAM strain capsid to be 180; our nucleotide sequence predicts 223. Because of this size difference the comparison presented in Table 2 is given in percent amino acids rather than in molar ratios.

The 5' and 3' Flanking Regions

The coding region is preceded by a 5' non-translated region of 573 nucleotides. The 5' most 68% of this tract consists of two sets of almost perfect direct repeats. The first repeat is 119 nucleotides long and is found from positions 1 to 119 and again from 119 to 237. The second repeat consists of the 76 nucleotides occupying positions 236 to 311 and 312 to 387. There are two nucleotide changes within each set of repeat units as indicated in Figure 2. An eleven nucleotide sequence of the second repeat at position 300 to 310 and 376 to 386 is repeated again from position 431 to 441 with a single nucleotide change. To confirm the existence of these repeat units, RNA-2 was used directly as template for the dideoxynucleotide sequencing reaction with two different oligomers as primers (Figure 1).

A partial sequence of RNA-1 was determined by partial enzymatic diges-



tion of 3' end labeled RNA-1 and by dideoxynucleotide sequencing using polyadenylated RNA-1 as template and (dT)$_8$dG as well as the synthetic oligomers complementary to the 3' region of RNA-2 (see Figure 1) as primers. RNA-2 and RNA-1 are exactly identical for the last 459 nucleotides at the 3' terminus (Figure 2). We have sequenced RNA-1 another 70 nucleotides upstream from the region of homology and have found no obvious similarity between this region and the corresponding region of RNA-2.

DISCUSSION

The sequence of CAM strain TRV RNA-2 has a number of interesting features. It contains a single long open reading frame which almost certainly codes for capsid protein. This conclusion is based on several lines of evidence. Primary among these is the absence of capsid protein synthesis and rod formation when RNA-2 is omitted from an inoculum. In addition, when an inoculum consists of long rods of one strain and short rods of another (pseudorecombinants) the resulting virus is encapsidated with protein specified by RNA-2 (25, 26, 27). It is also well established that the RNA-2 from CAM and some other strains of TRV serves as template for capsid protein in cell free translation systems (3, 4). Finally, the amino acid sequence of the protein deduced from the long open reading frame is similar to that determined by two other groups of investigators (24,25). It is possible that the difference in the number of total amino acids in capsid protein deduced from the nucleotide sequence (223) and that extrapolated by others from amino acid composition data (180) results from post-translational processing. If the protein undergoes such processing, it is likely to occur at the amino terminus but not at the carboxy terminus because five of the last seven residues in the predicted sequence are prolines. A similar proline rich carboxy terminus has been observed in a Dutch strain of TRV (1). It is also possible that the molecular weight of the protein is larger than the 22,000 estimated from amino acid composition studies (24, 25). The molecular weight estimate of CAM strain capsid protein has been revised to 23,000

Fig. 2: The nucleotide sequence of TRV RNA-2 (CAM strain). Shown are the long untranslated 5' and 3' regions flanking the central open reading frame with its deduced 223 amino acid translation product. The first set of direct repeats in the 5' leader sequence is indicated by the thick followed by the thin underline. The second repeated sequence is shown by thick and thin dashed underlines. Lower case letters in the 5' region designate nucleotide differences between repeats. The 13 unused AUGs of 5' leader sequence are overlined. The region of the 3' terminus identical to that of TRV RNA-1 is indicated by the dotted underline.

Table 1: Nucleotide frequencies given as percentages.

	%	U	C	A	G
TRV RNA-2		32	18	26	24
Open Reading Frame		29	23	23	25
3rd Position of Codons		42	16	18	25

Table 2: Derived amino acid composition in percent of the CAM strain TRV RNA-2 open reading frame (ORF) compared with that of the capsid proteins of CAM strain and a closely related, if not identical, isolate (Z).

	TRV CAM capsid[1]	TRV CAM RNA-2 ORF	TRV Z capsid[2]
Ala	12.4	12.6	12.8
Arg	4.5	4.5	5.0
Asp	8.4	9.0	9.4
Cys	0.6	0.4	0.6
Glu	7.3	6.7	8.3
Gly	6.7	6.7	5.5
His	0.6	0.4	0.6
Ile	2.2	2.2	2.2
Leu	6.2	5.8	6.1
Lys	7.9	7.2	7.2
Met	1.1	1.3	1.1
Phe	5.6	4.9	5.0
Pro	10.1	8.1	7.7
Ser	11.8	13.9	12.2
Thr	5.6	6.3	6.1
Trp	1.1	1.3	1.1
Tyr	2.8	2.7	2.8
Val	5.1	5.8	6.1
N=	180	223	180

1) Calculated from mole ratio data presented in (26).
2) Calculated from mole ratio data presented in (27).

on the basis of its gel electrophoretic behavior (28), a value in closer agreement to the Mr 23,654 derived from the open reading frame.

The codon usage for the long open reading frame is presented in Table 3. Uridylic acid residues are strongly favored in third positions (Tables 1 and 3). For example, use of the six serine codons deviates significantly from that expected based on nucleotide frequency; 52% of all serine residue

Table 3: Codon distribution within long open reading frame of TRV RNA-2.

	U		C		A		G		
U	phe	6	ser	16	tyr	3	cys	1	U
		5		3		3		0	C
		2		3	---		---	1	A
		5		2	---		trp	3	G
C	leu	3	pro	7	his	0	arg	1	U
		1		2		1		2	C
		0		6	gln	4		0	A
		2		3		2		0	G
A	ile	5	thr	8	asn	4	ser	7	U
		0		1		1		0	C
		0		1	lys	9	arg	4	A
	met	3		4		7		3	G
G	val	6	ala	14	asp	6	gly	8	U
		2		4		9		1	C
		1		3	glu	3		3	A
		4		7		6		3	G

are encoded by UCU, whereas only 37% would be expected on a random basis. Likewise, 50% of all alanine residues are encoded by GCU. In general the percentage of U in third positions is 42% and is 52% among the four-fold degenerated codons. This value is higher than the 43.4% observed in such positions in the long open reading frame of cowpea mosaic virus M component, previously noted as being higher than most eucaryotic or eucaryotic viral mRNAs (29). The preponderance of uridines in the third position does not continue in the 5' or 3' flanking region in-frame triplets. Uridines are present in the third positions of the coding sequence 1.47 times more frequently than expected, as compared to 1.03 times and 0.93 times the expected frequency in the 5' and 3' flanking regions, respectively. The preferred use of U in the third position of four-fold degenerated codons has been correlated with highly expressed procaryotic cistrons (30).

Two methionine codons lie at the beginning of the open reading frame at positions 574 and 580. It is likely that the first AUG initiates translation of capsid because it and not the second one looks like the typical initiating codon in being flanked by a purine (preferably A) at the -3 position and a G at the +4 position (31). The long 573 nucleotide leader

sequence 5' to the open reading frame contains 13 other AUG codons, four of which lie within the same frame as the coat protein cistron. This is similar to the leader sequences found in the picornavirus group. Human rhinovirus type 14, for example, also has 13 apparently unused AUG codons within its 677 nucleotide leader sequence, four of which are in frame (32). Of the 13 AUGs present in the leader of TRV RNA-2 only one could initiate translation of a polypeptide of more than 15 amino acids. This AUG, at position 482, begins an open reading frame which overlaps that of the coat protein cistron by 97 nucleotides and could code for a polypeptide of 63 amino acids. It is not known whether this short open reading frame is of biological significance, however its AUG flanking sequence does not conform to that of other initiating AUGs (31).

The presence of the multi-AUG leader raises the question of how the initiating AUG at position 574 is recognized. It seems unlikely that only the flanking sequence determines initiation because several of the leader AUGs have purines at postions -3 and +4 and one has the preferred A at -3 and G at +4. This one, however, can translate only a 13 residue polypeptide. Perhaps RNA tertiary structure is important in highlighting the AUG at the beginning of the open reading frame. Alternatively, it is possible that RNA-2 is not the _in vivo_ messenger; capsid protein may be translated from a subgenomic RNA. Infected plant tissue does contain a subgenomic RNA (Mr 0.5×10^6) generated from RNA-2 (5) which is long enough to contain the capsid protein cistron and act as the _in vivo_ mRNA. Some other TRV strains have an RNA-2 much longer than that of the CAM strain. SYM strain RNA-2 (Mr 1.36×10^6) is almost twice as long and it proves not to be an efficient _in vitro_ mRNA for capsid protein. Capsid is, instead, translated from a smaller RNA (Mr 0.6×10^6), which is presumably a subgenomic RNA generated from RNA-2 (33).

The 5' leader sequence consists primarily of the two sets of direct repeats. The 5' terminus has been reported to serve as the encapsidation initiation site for TRV (34), and what role, if any, a repeat of this sequence might have is unknown.

Our finding that the 3' termini of RNA-1 and RNA-2 are identical over a span of 459 nucleotides confirms and extends the results of hybridization studies which showed that the two CAM strain RNAs have a 450 to 600 nucleotide homology (23, 35, 36). The 3' terminus probably serves as a replicase recognition site. Thus, it is not surprising that, as is true for the RNAs of other multipartite plant viruses, the 3' termini are similar. RNAs 1, 2,

and 3 of brome mosaic virus, for example, are homologous over the last 180 nucleotides, with differences at only four positions (37). Homology is also present, but less strong, between the B and M RNAs of cowpea mosaic virus, where there is 80% homology between the last 65 nucleotides adjacent to the poly (A) tracts (38). The fact that CAM strain TRV RNAs are identical over such a long region indicates either strong selection pressure resulting from a remarkable stringency for a functional base sequence or, perhaps, a form of communication between the two RNAs, possibly recombination, which serves to maintain identity in a critical region. It is difficult to imagine the nature of either mechanism without additional information, particularly when the strong identity observed in the CAM strain is apparently not found in all strains of TRV. No homology has been detected in hyridization studies between the two RNAs of either the SYM or the OR stains of TRV (35).

The organization of TRV RNA-2 with the unusually long 5' and 3' untranslated regions flanking a long open reading frame is not unique. A similar structure has recently been reported for both RNAs 3 and 4 of beet necrotic yellow vein virus (39). Such 5' and 3' untranslated regions may prove to be even longer in other strains of TRV because they have larger RNA-2s than does the CAM strain, and it seems doubtful that they would have additional cistrons. Besides roles in replicase recognition and possibly encapsidation initiation, these long termini may also function in maintaining stability of the RNA or in regulating in a temporal fashion the amount of capsid protein synthesized.

ACKNOWLEDGMENTS

This work was supported in part by grants from NIH (GM 27064) and USDA (CRCR-1-0607). S.T.B. received support from a Wayne State University Graduate Fellowship. R.A.T. was supported by the NIH MBRS program (RR-08167).

Present addresses: [1]Rohm and Haas Co., Spring House, PA 19477 and [2]Department of Pharmacology, Yale University, New Haven, CT 06510, USA

*To whom correspondence should be addressed

REFERENCES

1. Harrison, B.D. and Robinson, D.J. (1978) Advan. Virus Res. 23, 25-77.
2. Pelham, H.R.B. (1979) Virology 97, 256-265.
3. Mayo, M.A., Fritsch, C., and Hirth, L. (1976) Virology 69, 408-415.
4. Fritsch, C., Mayo, M.A., and Hirth, L. (1977) Virology 77, 722-732.

5. Bisaro, D. and Siegel, A. (1980) Virology 107, 194-201.
6. Christie, S.R. (1969) Plant Dis. Rep. 53, 939-941.
7. Hari, V. (1981) Virology 112, 391-399.
8. Bailey, J.M. and Davidson, N. (1976) Anal. Biochem. 70, 75-85.
9. Sippel, A. (1973) Eur. J. Biochem. 37, 31-40.
10. Land, H., Grez, M., Hauser, H., Lindenmaier, W., and Schütz, G. (1981) Nuc. Acid Res. 9, 2251-2266.
11. Wickens, M.P., Buell, G.N., and Schimke, R.T. (1978) J. Biol. Chem. 253, 2483-2495.
12. Crouse, G., Frischauf, A., and Lehrach, H. (1983) Methods Enzymol. 101, 78-89.
13. Gergen, J.P., Stern. R.H., and Wensink, P.C. (1979) Nuc. Acid Res. 7, 2115-2136.
14. Taylor, J.M., Illmensee, R., and Summers, J. (1976) Biochim. Biophys. Acta 442, 324-330.
15. Tautz, D. and Renz, M. (1983) Anal. Biochem. 132, 14-19.
16. Messing, J. (1983) Methods Enzymol. 101, 20-78.
17. Biggin, M.D., Gibson, T.J., and Hong, G.F. (1983) Proc. Natl. Acad. Sci. USA 80, 3963-3965.
18. Larson, R. and Messing, J. (1982) in The Applications of Computers to Research on Nucleic Acids, Söll, D. and Roberts, R.J., Eds., pp. 39-43, IRL Press, Oxford.
19. England, T.E., Bruce, A.G., and Uhlenbeck, O.C. (1980) Methods Enzymol. 65, 65-74.
20. D'Alessio, J.M. (1982) in Gel Electrophoresis of Nucleic Acids: a Practical Approach, Rickwood, D. and Hames, B.D., Eds., p. 173-197, IRL Press, Oxford.
21. Kemper, B. (1976) Nature 262, 321-323.
22. Alquist, P., Dasgupta, R., Shih, D., Zimmern, D., and Kaesberg, P. (1979) Nature 281, 277-282.
23. Minson, A.C. and Darby, G. (1973) J. Gen. Virol. 19, 253-262.
24. Miki, T. and Okada, Y. (1970) Virology 42, 993-998.
25. Ghabriel, S.A. and Lister, R.M. (1973) Virology 52, 1-12.
26. Sänger, H.L. (1968) Mol. Gen. Genet. 101, 346-367.
27. Sänger, H.L. (1969) J. Virol. 3, 304-312.
28. Mayo, M.A. and Robinson, D.J. (1975) Intervirology 5, 313-318.
29. van Wezenbeek, P., Verver, J., Harmsen, J., Vos, P., and van Kammen, A. (1983) The EMBO J. 2, 941-946.
30. Grantham, R., Gautier, C., Gouy, M., Jacobzone, M., and Mercier, R. (1981) Nuc. Acid Res. 9, r43-r74.
31. Kozak, M. (1981) Nuc. Acid Res. 9, 5233-5252.
32. Callahan, P., Mizutani, S., and Colonno, R.J. (1985) Proc. Natl. Acad. Sci USA 82, 732-736.
33. Robinson, D.J., Mayo, M.A., Fritsch, C., Jones, A.T., and Raschké, J.H. (1983) J. Gen. Virol 64, 1591-1599.
34. Abou Haidar, M. and Hirth, L. (1977) Virology 76, 173-185.
35. Robinson, D.J. (1983) J. Gen. Virol. 64, 657-665.
36. Darby, G. and Minson, A.C. (1973) J. Gen. Virol. 21, 285-295.
37. Alquist, P., Dasgupta, R., and Kaesberg, P. (1981) Cell 23, 183-189.
38. Davies, J.W., Stanley, J., and van Kammen, A. (1979) Nuc. Acid Res. 7, 493-500.
39. Bouzoubaa, S., Guilley, H., Jonard, G., Richards, K., and Putz, C. (1985) J. Gen. Virol 66, 1553-1564.